flask. Dissolve and dilute to volume with distilled water and mix.

(2) *Buffer solution, pH 7.0.* Transfer 5.68 grams of sodium phosphate, dibasic USP and 3.63 grams of potassium phosphate monobasic to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(3) *Mobile phase.* Mix buffer solution, pH 3.6: acetonitrile (9:1). Filter through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(4) *Internal standard solution.* Transfer 1.2 grams of salicylic acid to a 200-milliliter volumetric flask. Dissolve in 10 milliliters of methyl alcohol, dilute to volume with buffer solution, pH 7.0, and mix.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of working standard and sample solutions*—(1) *Working standard solution.* Place approximately 50 milligrams of cefazolin working standard, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(2) *Sample solution.* Place approximately 50 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution prepared as directed in paragraph (d)(1) of this section into the chromatograph. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (d)(2) of this section into the chromatograph and repeat the procedure described for the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components. The elution order is void volume, salicylic acid and cefazolin.

(f) *Calculation.* Calculate the micrograms of cefazolin per milligram of sample as follows:

$$\text{Micrograms of cefazolin per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

R_u = Area of the cefazolin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard) /Area of internal standard peak;

R_s = Area of the cefazolin peak in the chromatogram of the cefazolin working standard/Area of internal standard peak;

P_s = Cefazolin activity in the cefazolin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

[48 FR 33478, July 22, 1983; 48 FR 34947, Aug. 2, 1983]

§ 436.343 High-pressure liquid chromatographic assay for cefuroxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 15-centimeter column having an inside diameter of 4.6 millimeters and packed with hexyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers in diameter.

(b) *Reagents*—(1) *Acetate buffer, pH 3.4.* Place 50 milliliters of 0.1*M* sodium acetate into a 1,000-milliliter volumetric flask and dilute to volume with 0.1*M* acetic acid. Mix.

(2) *Mobile phase.* Mix 0.1*M* acetate buffer, pH 3.4:acetonitrile (10:1). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(3) *Internal standard solution.* Prepare a 1.5 milligram per milliliter solution of orcinol monohydrate in water.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the cefuroxime working standard with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours.

(2) *Preparation of sample solutions*—(i) *Product not packaged for dispensing (micrograms of cefuroxime per milligram).* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(ii) *Product packaged for dispensing.* Determine both micrograms of cefuroxime per milligram of the sample and milligrams of cefuroxime per container. Use separate containers for preparation of each sample solution as described in paragraphs (d)(2)(ii) (*a*) and (*b*) of this section.

(*a*) *Micrograms of cefuroxime per milligram.* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(*b*) *Milligrams of cefuroxime per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with distilled water to obtain a stock solution of 1.0 milligram per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (d)(2)(i) of this section into the chromatograph and repeat the procedure

described for the working standard solution. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (d)(2)(ii)(*a*) and (d)(2)(ii)(*b*) of this section.

(f) *Calculations.* (1) Calculate the micrograms of cefuroxime per milligram of sample as follows:

$$\text{Micrograms of cefuroxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) Calculate the cefuroxime content of the vial as follows:

$$\text{Milligrams of cefuroxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter; and

d= Dilution factor of the sample.

[48 FR 38460, Aug. 24, 1983; 48 FR 40704, Sept. 9, 1983]

§ 436.344 Thin layer chromatographic identity test for cefuroxime.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel F or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform, methanol, and formic acid in volumetric proportions of 90:16:4, respectively.

(c) *Preparation of the spotting solutions.* Dissolve approximately 200 milligrams each of the working standard and sample in 5 milliliters of a 50 percent aqueous acetone solution.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot 5 microliters each of the sample and working standard solutions. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel a minimum of 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry. Observe under ultraviolet light (254 nanometers).

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[48 FR 38461, Aug. 24, 1983]

§ 436.345 High-pressure liquid chromatographic assay for ceftizoxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and